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AD846832

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Army Biological Defense Research Lab ltr dtd 13 Sep 1971

TRANSLATION NO. 254

DATE: July 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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Properties of an infectious fraction of nucleic acid from chicken embryos infected with the virus of encephalitis. First Report: Physical and chemical properties.

by E. Wecker.

Zschr. f. Naturforschg. 14 b, 370-378 (1959). (Only designated portions have been translated).

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2. Viscosity.

When the density is known, two additional parameters are required for the determination of molecular weight, e.g. the viscosity factor and the sedimentation constant. By utilizing these two parameters, Gierer has established the molecular weight of infectious ribonucleic acid (RNA) from tobacco mosaic virus (TMV), (1).

Similar investigations of the infectious nucleic acid (NA) fractions discussed here were indicated.

The viscosity factor $[\eta]$ was related to the volume of dissolved nucleic acid; it is defined in dimensionless units according to the following equation:

$$[\eta] = \frac{\varphi}{c}\left(\frac{\eta}{\eta_0} - 1\right)$$

(φ = density; c = concentration of NA; η = viscosity at a concentration c in g/l cm^3; η_0 = viscosity of the pure solvent).

An NA fraction precipitated from alcohol, with a concentration of 0.86 mg/cm^3, yielded a viscosity factor of $[\eta]$ = 1120 computed for this NA concentration, i.e. about 10-fold viscosity of an RNA from TMV of equal concentration (1). It could be expected, therefore, that other, more viscous substances were present in the NA fractions, in addition to RNA.

The contribution of the RNA portion to the total viscosity of an NA fraction was determined as follows: After the passage time of an NA fraction freshly precipitated from alcohol was ascertained in the viscosimeter, the preparation was incubated with RNase (1 γ/cm^3) for 30 minutes at 37°C and again measured. The passage time had decreased from the original 498 sec. to 481.7 sec., and the relation V of the corresponding viscosities was V = 0.967. Subsequently the preparation was incubated with DNase (1 γ/cm^3) for 30 minutes at 37°C, resulting in another drop in passage time (V = 0.822).

It follows from these results that a considerable portion of the total internal viscosity of the NA fraction is due to DNA.

After exhaustive treatment of the preparation with RNase and DNase, the passage time nevertheless was still 80 sec. longer than that of the pure solvent.

Since the internal viscosity of degraded nucleic acid molecules may be neglected, an additional substance had to be present in the NA fraction, other than RNA and DNA (cf. chemical properties, chapter II).

The percentual decrease in viscosity achieved by means of enzymic cleavage of NA molecules of high weight yields a direct measure for the viscosity contribution of both types of heavy-molecular nucleic acids.

The viscosity factor $[\eta]$ of RNA or DNA could then be computed from the chemically established, actual DNA or RNA concentrations of the preparation.

That of the RNA portion at $[\eta] = 95$ is of the same magnitude as that ($[\eta] = 100$) found by Gierer in the case of, e.g. RNA from microsomes of rat livers (2). The viscosity factor $[\eta]$ of DNA at 1130 is considerably higher, as expected (see Table 2).

	Viscosity factor $[\eta]$
Total NA (0.86 mg/cm^3)	1120
RNA (0.54 mg/cm^3)	95
DNA (0.32 mg/cm^3)	1130

Table 2. Viscosities.

3. Sedimentation constants.

Examination of NA fractions in the analytical ultracentrifuge reveals different gradients.

The relation of crude and alcohol-precipitated NA fractions shall be discussed separately, since the alcohol-precipitation changes the relation RNA/DNA and thereby the entire internal viscosity of the solution. The following gradients were observed in connection with the crude NA fractions: A rapidly moving gradient A with $s_{20} \sim 32$ S, a slowly moving gradient with $s_{20} \sim 12$ S. s_{20} of gradient B found in one case lies between those of A and C.

The following values were found in the case of NA fractions precipitated from alcohol:

gradient A $s_{20} \sim 30$ S
gradient B $s_{20} \sim 19$ S
gradient C $s_{20} \sim 10$ S.

The measured sediment values depend upon the concentration (see Table 3). The values given above were extrapolated graphically to infinitely small concentrations of NA.

The sedimentation constants measured individually for their dependence upon the concentration in the case of both alcohol-precipitated and crude NA fractions are listed in Table 3.

Preparation	Total NA mg NA/cm^3	s_{20} gradient A	s_{20} gradient B	s_{20} gradient C
Crude NA fraction	1.86	25.5	15.3	7.8
	1.94	25.0		4.9
	2.84	21.8		6.0
Alcohol-precipitated NA fraction	0.95	23.7		8.2
	1.54	21.4		8.4
	1.64	21.4	14.2	7.95
	2.88	13.5	9.5	1.25
	2.90	14.5	10.9	4.85

Table 3. The sedimentation constants of the gradients of crude and alcohol-precipitated fractions of NA.

The values found for gradients A and B agree with those measured by Gierer for RNA from microsomes (2). Moreover, the sedimentation constant of gradient A coincides with that found by Gierer for RNA from TMV (1).

Data on the chemical nature of the substances deposited in three visible gradients were obtained by the following experiment:

An alcohol-precipitated NA fraction with 2.1 mg NA/cm^3 was examined in the analytical ultracentrifuge. It showed the three typical gradients with the sedimentation constants s_{20} A = 19.4 S; s_{20} B = 12.6 S; s_{20} C = 6.4 S (see Fig. 2).

A sample of the same NA fraction was treated with 1 γ RNase/cm^3 for 10 minutes at room temperature. It was found upon subsequent centrifugation that the gradients A and B had completely disappeared. The gradient C, its size unaltered, had a sedimentation constant s_{20} = 7.9 S (see Fig. 3).

When, on the other hand, the material was pre-treated with 1 γ DNase/cm^3 for 60 minutes at room temperature, the gradients A and B remained optically unchanged and sedimented with s_{20} A = 21.5 S and s_{20} B = 11.4 S. Gradient C was considerably flattened and widened thereby, even though it did not disappear entirely (see Fig. 4).

Thus gradients A and B are due to RNA, gradient C to DNA. The latter is subject to a strong self-intensifying effect owing to the high viscosity of the material. If the relative concentration of the substances is computed from the surface area of the three gradients, about 70% is obtained for A, 5% for B and 25% for C.

In another test an alcohol-precipitated NA fraction was mixed with 1.9 mg NA/cm^3, and an RNA from TMV with 1.3 mg RNA/cm^3 at equal parts.

Examination of this mixed preparation in the analytical ultracentrifuge failed to reveal additional gradients, other than A, B and C, even upon extended centrifugation. The surface area of gradient A was enlarged, however. This result also indicates that RNA A of the NA fraction and RNA from TMV have the same sedimentational properties.

Since the behavior of the NA fraction in the analytical ultracentrifuge was known, it remained to clarify the question whether the infectious principle could be attributed to one of the three gradients observed.

An attempt was made, therefore, to determine the sedimentation constant of the infectious principle of the NA fraction from the drop in infectiosity after high-speed centrifugation of the material.

Various experiments were conducted in this connection: In one test series the sedimentation constants of the three different gradients of a certain NA fraction were measured optically. At the same time 4 cm^3 each of the same material was centrifuged at 40,000 rpm in rotor 40 of the Spinco centrifuge for 55 and 60 minutes at $\pm$ 5°C, and the decrease in infectiosity of the supernatants compared to that of the starting material was determined in the egg test (dilutions by powers of 5, 10 eggs/dilution). From the known sedimentation constant of the three gradients and the centrifugational data used in connection with preparative centrifugation it was possible to compute with sufficient accuracy the percentage of the individual substances that must have sedimented from the supernatant (3). As Table 4 shows, the comparison of these values with those found in the infective tests reveals relatively close agreement of the biologically determined sedimentational properties with those of gradient A.

In a similar test 1.5 cm^3 of an NA fraction was centrifuged in an oscillating rotor of the Spinco centrifuge for 130 minutes at 39,000 rpm. The temperature at the end of centrifugation amounted to $\pm$ 18°C. The upper 2/3 were then drawn off carefully and tested in the infective test.

The result is also shown in Table 4. Here, too, favorable agreement of the measured values with those computed for gradient A is evident.

Finally, in two tests a fraction of NA was centrifuged in an analytical ultracentrifuge until gradient A was completely sedimented. 80% of the total supernatant was then carefully removed and, due to the

small volume, only qualitatively tested for infectiosity. The same procedure was effected with the supernatant after 70% sedimentation of gradient A. The values obtained in this connection are as follows:

	sedimented (%)		infectiosity of the upper 80% of the supernatant
	A	B	
1.	100	70	negative
2.	70	40	positive

All these attempts at biological determination of sedimentation constants of the infectious principle attributed to NA fractions reveal a factor that is within the order of magnitude established for RNA gradient A. From this, with due regard for the viscosity factor of the RNA portion of the NA fraction, a probable molecular weight of about $2 \cdot 10^6$, within the limits of $1 \cdot 10^6$ and $3 \cdot 10^6$, is obtained for the infectious component.

Illustrations

Fig. 2-4: Sedimentation diagrams of an alcohol-precipitated NA fraction. Fig. 2: Not treated with enzyme. Fig. 3: After treatment with RNase. Fig. 4: After treatment with DNase.

Literature

(1) A. Gierer, Z. Naturforschg. 13 b, 477 (1958). (2) A. Gierer, Z. Naturforschg. 13 b, 788 (1958). (3) P. Giebler, Z. Naturforschg. 13 b, 238 (1958).

Preparation	Decrease (%) analytical UC(*)		theoretical loss of infectiosity		actual loss of infectiosity	deviations (%)	
	$s_{20}A$	$s_{20}B$	A	B		to A	to B
4 cm^3 40,705 rpm 55 min. ≠5°C 2.9 mg NA/cm^3	13.5	9.5	38	19	30	- 8	≠11
4 cm^3 39,500 rpm 60 min. ≠5°C 2.1 mg NA/cm^3	19.4	12.6	63	35	92	≠29	≠57
4 cm^3 40,800 rpm 60 min. ≠5°C 0.86 mg NA/cm^3	26	16.4	83	48	96	≠13	≠48
1.5 cm^3 39,000 rpm 130 min. 18°C 2.1 mg NA/cm^3 oscillating rotor	19.4	12.6	100	86	96	- 4	≠10
Average						≠ 7.5	≠31.5

(*) UC = ultracentrifugation

Table 4. Behavior of the gradients A and B and the infectiosity of NA fractions upon centrifugation.